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Cognitive Performance in Older Adults Is Inversely Associated with Fish Consumption but Not Erythrocyte Membrane n–3 Fatty Acids[1–3]

Vanessa Danthiir,[4]* Diane Hosking,[4] Nicholas R. Burns,[5] Carlene Wilson,[4,7] Ted Nettelbeck,[5] Eva Calvaresi,[4] Peter Clifton,[4,8] and Gary A. Wittert[6]

[4]Commonwealth Scientific and Industrial Research Organisation, Preventative Health Research Flagship, Animal, Food and Health Sciences, Adelaide, Australia; [5]School of Psychology, and [6]School of Medicine, University of Adelaide, Australia

Abstract

Higher n–3 (ω-3) polyunsaturated fatty acids (PUFAs) and fish intake may help maintain cognitive function in older age. However, evidence is inconsistent; few studies have examined the relation in cognitively healthy individuals across numerous cognitive domains, and none to our knowledge have considered lifetime fish intake. We examined associations between multiple domains of cognition and erythrocyte membrane n–3 PUFA proportions and historical and contemporary fish intake in 390 normal older adults, analyzing baseline data from the Older People, Omega-3, and Cognitive Health trial. We measured n–3 PUFA in erythrocyte membranes, and we assessed historical and contemporary fish intake by food-frequency questionnaires. We assessed cognitive performance on reasoning, working memory, short-term memory, retrieval fluency, perceptual speed, simple/choice reaction time, speed of memory-scanning, reasoning speed, inhibition, and psychomotor speed. Cognitive outcomes for each construct were factor scores from confirmatory factor analysis. Multiple linear regression models controlled for a number of potential confounding factors, including age, education, sex, apolipoprotein E-ε 4 allele, physical activity, smoking, alcohol intake, socioeconomic variables, and other health-related variables. Higher erythrocyte membrane eicosapaentonoic acid proportions predicted slower perceptual and reasoning speed in females, which was attenuated once current fish intake was controlled. No other associations were present between n–3 PUFA proportions and cognitive performance. Higher current fish consumption predicted worse performance on several cognitive speed constructs. Greater fish consumption in childhood predicted slower perceptual speed and simple/choice reaction time. We found no evidence to support the hypothesis that higher proportions of long-chain n–3 fatty acids or fish intake benefits cognitive performance in normal older adults. J. Nutr. 144: 311–320, 2014.

Introduction

There is considerable interest in the potential of n–3 PUFA intake, particularly long-chain n–3 PUFAs of marine origin (DHA and EPA), to maintain cognitive function in older age (1). Associations with cognitive outcomes have been examined in terms of dietary intake of n–3 FAs and fatty fish, relatively high in long-chain n–3 PUFAs (particularly oily fish), or n–3 PUFA concentrations in blood, with a lack of consistent findings.

In prospective studies, incident dementia and cognitive decline have been have been shown to be inversely related to dietary fish intake (2–5) as well as to biological markers of long-chain PUFA status (6–9). In contrast, other prospective studies have not shown such associations (10–13).

Fewer studies have reported cross-sectional associations between n–3 PUFA or fish intake and normal cognitive functioning in older age. Higher fish intake has been positively related to cognitive outcomes (14,15), but this is not a consistent finding. Dietary measures of FAs and fatty fish intake were not related to baseline cognitive performance in the Veterans Affairs Normative Aging Study and there was a trend toward an inverse relation between fatty fish and a measure of memory and language after adjustment for confounding factors (13).

Studies that measure n–3 PUFAs in plasma or RBCs may provide more reliable evidence of associations between n–3

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[7] Present address: Centre for Innovation in Cancer, Flinders University, and Cancer Council South Australia, Adelaide, Australia.
[8] Present address: Baker IDI Heart and Diabetes Institute, Adelaide, Australia.
* To whom correspondence should be addressed. E-mail: vanessa.danthiir@csiro.au.

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PUFA status and cognition; however, few studies have reported such associations in older, cognitively nonimpaired individuals. In 2 studies, one found no associations between plasma long-chain PUFA concentrations and cross-sectional performance on 5 measured cognitive domains (9), whereas the other, much smaller study found total n–3 PUFAs measured in erythrocyte membranes were associated with cognitive performance on a single cognitive factor derived from 6 tests (16).

Differences in methods of measurement of n–3 PUFA status and the inclusion or exclusion of covariates (17), in particular carrier status of the apoE-ε4 allele (16), may contribute to the heterogeneity of reported outcomes. Differences in the assessment of cognitive performance in particular are a major impediment to meaningful comparisons among studies (18). Cognition is multidimensional, with domains that are subject to differential change across the lifespan (19), yet the majority of studies focus on a global measure of cognition that cannot uncover the relation between n–3 PUFAs and specific cognitive domains.

A particular challenge when assessing nutritional contributions to individual differences in older-age cognition is that in addition to current intake, long-term dietary intake may be of relevance (20). Dietary intake is not static over time and changes have been demonstrated for both dietary pattern scores (21,22) and nutrient intake (23). Because the potentially relevant period of exposure for older-age cognition stretches over multiple decades (24,25), assessing historical fish consumption in conjunction with more recent dietary measures would therefore be informative when examining n–3 PUFA and fish intake associations with older-age cognition. To our knowledge, such an analysis has not been previously reported.

The current study examines associations between contemporary erythrocyte membrane n–3 FA proportions and fish intake as well as historical fish intake, with cognitive performance measured across multiple domains, controlling for a comprehensive array of covariates including the apoE-ε4 allele.

Materials and Methods

This study presents baseline data from the Older People, Omega-3, and Cognitive Health (EPOCH)[9] Trial, a double-blind, randomized, placebo-controlled, parallel trial examining the effect of 18 mo of long-chain n–3 FA (DHA-rich) supplementation on cognitive decline in older adults. Full methodological details can be found in the trial protocol (26). All experimental procedures were approved by the Human Research Ethics Committee of the Commonwealth Scientific Industrial and Research Organisation; the study was conducted in compliance with the Declaration of Helsinki and followed Good Clinical Research Practice Guidelines. Written informed consent was obtained from all participants.

Participants

Participants were community-dwelling older adults living in Adelaide, South Australia. Eligibility requirements were age 65–90 y and fluency in English. Exclusion criteria were taking n–3 fish-oil (or algal) supplements, any condition where cognitive impairment may be a consequence, serious medical conditions or medication dosages known to interfere with cognition, current major clinical depression, diabetes, dementia, or a score <22/27 on a telephone-administered version (27) of the Mini-mental State Examination (equivalent to <24/30 on the standard Mini-mental State Examination, the traditional cutoff for possible dementia).

Three hundred and ninety eligible people (46.4% male) aged 65–91 y [mean ± SD = 73.1 ± 5.5] completed baseline assessment; of these, 21.8% had completed a high school education and 41.5% had additional tertiary qualifications.

Procedure

Participants completed a battery of psychological, health, lifestyle, and demographic questionnaires 2 wk prior to their baseline assessment, for which they attended the center in the morning after an overnight fast. A venous blood sample and physical measurements (height, weight, blood pressure) were taken by trained staff and participants then ate a standardized breakfast before completing the cognitive test battery of computerized and paper-and-pencil tests. The testing session lasted about 4 h, with breaks of ~10 min every hour, and was completed in groups (maximum 7 participants) supervised by 2 trained research staff. Three months after baseline assessment, participants 352 (90%) completed a historical FFQ.

Measures

Cognitive tests. The test battery was designed to be sensitive to subtle differences in performance that might arise from nutritional differences. Details of the test battery's design and composition were previously fully described (26). Briefly, a vocabulary task (a marker of crystallized intelligence) was administered and the following constructs were assessed by 2 or more tests (in parentheses): perceptual speed (finding as, number comparison, digit-symbol coding); simple/choice reaction time (simple, 2-choice, 4-choice); speed of memory-scanning (number and letter memory scanning); reasoning speed (number and letter odd-man-out); inhibition (Simon task, spatial and color Stroop); psychomotor speed (simple, up, and diagonal movement tasks); reasoning (Raven's standard progressive matrices, letters sets, everyday problem solving); working memory (operation span, counting span); short-term memory (face recognition, word list recall, paired associates); and retrieval fluency (word endings, categories).

n–3 FA analysis. Three long-chain n–3 FAs, DHA, EPA, and docosapentaenoic acid (DPA), as well as total n–3 were measured in erythrocyte membranes by GC, as previously described (26) and expressed as a percentage of total FAs.

Fish consumption frequency. Current fish consumption frequency was derived from a previously validated PUFA FFQ (28). Historical fish intake was derived from the nonquantitative Lifetime Diet Questionnaire (LDQ) (24,25). In the PUFA FFQ, participants answered 2 questions about consumption frequency of canned, fresh, or frozen fish, respectively, with the frequency options of never, less than once/mo, 1–3 times/mo, once/wk, 2 times/wk, 3–4 times/wk, once/d, or ≥2 times/d. They also specified up to 4 types regularly consumed. To assess past fish intake in the LDQ, participants answered how frequently they consumed *1*) salmon, trout, sardines, mackerel, anchovies, herring, pilchards, or fresh tuna (i.e., fatty fish) and *2*) other fish (including canned tuna and takeaway) for the respective life periods of childhood, early adulthood, adulthood, and middle-age. Frequency options were daily, 2–3 times/wk, 2–3 times/mo, and rarely or never.

Other variables. Demographic data collected were age, sex, years of education (school and tertiary/vocational), native language (dichotomized as English or other), current income level (following the Australian Census, 2006; a 19-category variable), early-life income level (4 categories representing parents' income level), medication usage for hypertension, elevated cholesterol or cardiovascular conditions, antidepressants, and/or sedatives (all transformed into dichotomous variables indicating usage or nonusage), and smoking history (calculated as pack-years). The Cancer Council Victoria (CCV) FFQ (29) was used to collect general dietary information, and physical activity was assessed by the Yale Physical Activity Survey (30). Depressive symptoms were assessed by the Centre for Epidemiology Studies Depression Scale (31). Height and weight (kg/m^2) were used to calculate BMI and systolic and diastolic blood pressure were measured. ApoE genotype, plasma homocysteine, glucose, and malondialdehyde), LDL cholesterol, glycated hemoglobin), and serum C-reactive

[9] Abbreviations used: CCV, Cancer Council Victoria; DPA, docosapentaenoic acid; EPOCH, Older People, Omega-3, and Cognitive Health; LDQ, Lifetime Diet Questionnaire.

protein concentrations were all assessed from blood samples. Full details on the assessment of these measures are described elsewhere (26).

Data screening and transformations

Cognitive variables. Full details of the treatment of the cognitive variables have been detailed elsewhere (26). The mean correct latencies for the reaction time tasks were inverted, excepting the inhibition tasks' difference scores, to normalize distributions (32). The difference scores for color Stroop and spatial Stroop were reflected and square root transformed, but the Simon task difference score was only reflected. Thus, speed scores for these 3 inhibition tasks are interpreted as smaller is better, reflecting less interference from irrelevant stimuli, whereas for all other speed task, a higher score reflects faster performance. The working memory tasks were transformed according to a transformation recommended for severe negative skewness (33) [NEWX = 1/(K − X) where X is the variable to be transformed and K is a constant from which each score is subtracted so that the smallest score is 1] and, along with the other accuracy based tasks, are interpreted as higher scores indicating better performance.

Other variables. Data were screened for univariate outliers; scores >3.29 SDs from the mean and disconnected from the main group were reduced to a value 1 unit from the next highest/lowest score within the main body of the distribution, to reduce their influence in subsequent analyses (33). For variables with a continuous underlying distribution, missing values (<2%) were imputed using Expectation Maximization estimation (34). Bivariate scatterplots of the outcomes with the independent FA or fish variables did not reveal any nonlinear relations.

ApoE genotype was transformed into a dichotomous variable representing apoE-ε4 allele carrier status; carriers were classified as any individual carrying the ε4 allele. The FA and continuous fish intake variables were centered, then multiplied, with the apoE-ε4 and sex variables, respectively, to calculate the interaction terms used in the analyses.

Fish consumption frequency. To determine contemporary fish consumption, canned and fresh/frozen fish consumption frequency, assessed by the PUFA FFQ, was transformed and combined to form a total weekly fish consumption variable. Fish types were categorized as oily (>2% fat when raw), following Hodge et al. (35) (based on NUTTAB 2010, Food Standards Australia and New Zealand, and Hodge et al.'s classifications), or white fish. Based on the number and types of fish specified, an estimate of the number of times per week oily or white fish was consumed was also calculated. (This calculation assumed equal consumption frequency for each type of fish that was specified by a participant.)

To determine historical fish consumption using the LDQ, historical total fish consumption frequency variables for each life period were calculated by transforming oily and white fish consumption into weekly frequency and combining the intake frequencies; a binary variable was formed representing infrequent consumption (rarely/never and 2–3 times/mo) compared with regular consumption (once per week or more). Due to multicollinearity issues in the initial analyses, the binary variables were coded as −0.5 (no/irregular consumption) and 0.5 (regular consumption). An alternative 3-category variable representing total past fish intake for each life period was also computed, with the categories of infrequent consumption (≤2–3 times/mo), 1–1.5 times/wk, and ≥2 times/wk.

Statistical analyses

Measurement models for the cognitive variables. Confirmatory Factor Analytic models were estimated using AMOS v7 (36) for the speed- and accuracy-based tasks separately, to derive factor scores representing the latent cognitive constructs to use in subsequent analyses, thereby providing a more reliable and valid measure of the cognitive constructs as opposed to individual task scores. Note that knowledge was indicated by only one task, with the residual variance specified as [(1-reliability)*VAR], and was included only for completeness of the model; scores representing this task were not used in subsequent analyses. Details of these models are presented as **Supplemental Text**. Factor scores were estimated for each construct with more than one indicator, based on the coefficients from these models, and used in the following analyses.

Correlational analysis. To examine the validity of the current fish consumption frequency variables, Pearson correlations were used to estimate associations between the fish variables and proportions of n–3 FAs.

Linear regression models. Multiple linear regression models examined associations between erythrocyte membrane n–3 FAs and contemporary and historical fish consumption frequency with the cognitive constructs. Cognitive outcomes were the factor scores for each construct.

Each FA/fatty fish intake predictor was first entered into a separate regression model. Each analysis controlled for age, sex, apoE-ε4 carrier status, and years of education. For each model, covariates were included that correlated with both the relevant cognitive variable and FA/fatty fish variable at $P < 0.10$ (model 1). The list of additional candidate covariates was smoking pack-years; English as a native language; current income level; early-life income level (for the childhood and early-adulthood historical fish intake models); daily alcohol intake (g/d; CCV FFQ); daily energy intake (MJ/d; CCV FFQ); plasma α- and β-carotene; serum vitamin B-12 and folate; vigorous activity level; medication for hypertension, cholesterol, or cardiovascular conditions; and antidepressants and/or sedatives.

Model 2 additionally incorporated either the apoE-ε4 by main predictor (fish intake/FA variable) interaction term or the sex by main predictor interaction term (without the apoE-ε4 interaction) to examine any potential interactions with apoE-ε4 carrier status or sex. If a significant interaction was observed, the significance of the individual slopes for each category of the relevant binary variable (sex or apoE-ε4 carrier status) was also assessed.

An additional step (model 3) was included when examining fish consumption frequency variables, without incorporating interaction terms. For the historical fish intake models, current total fish consumption and historical total fish consumption variables from the other life periods were also controlled. For the current fish intake models, historical total fish consumption for each life period was also controlled.

Fish intake could be a confounding factor in the FA regressions, in that fish is a source of exposures (other than long-chain n–3 PUFAs) that are relevant to cognition, both potentially beneficially [e.g., selenium (37)] or detrimentally [e.g., methylmercury (38)]. Therefore, current total weekly fish consumption frequency was entered as an additional covariate to model 1 (and model 3).

As a final step, in the models that featured significant associations between the FA/fatty fish variable and cognitive outcomes, covariates considered representing potential mechanisms of the relation were entered, again specifically tailored for each model with a correlation of $P < 0.10$ as the inclusion criterion; this step was included to determine whether the covariates attenuated the relation, indicating a potential mediating effect. This step was not conducted for the historical fish consumption models, because the potential mechanistic covariates were measured in the present and not considered likely to be modified by historical intake. The potential mechanistic variables were systolic and diastolic blood pressure (39,40), depressive symptoms (41,42), plasma homocysteine (43,44), malondialdehyde (45,46), LDL cholesterol (47,48), C-reactive protein concentrations (49,50), BMI, glucose, and glycated hemoglobin (51–54).

The covariates included in each model can be seen in **Supplemental Table 1**.

All regression models were conducted using IBM SPSS Statistics v20.0 (55). A P value < 0.05 (2-tailed) defined significance.

Results

Table 1 shows the descriptive statistics for the sample.

Associations between n–3 FA status and current fish intake. Pearson correlations found weekly consumption of fish (total) and oily fish were overall moderately related to erythrocyte

TABLE 1 Baseline characteristics of the EPOCH trial participants[1]

Variable	Value	Participants missing data, *n*
Age, *y*	73.1 ± 5.5	0
Sex, *% females*	53.6	0
Education, *y*	12.9 ± 4.2	10[2]
MMSE score	28.7 ± 1.3	2
Hypertensive medication used, *%*	41.3	0
Cardiac medication used, *%*	26.7	0
Cholesterol medication used, *%*	26.7	0
Antidepressant or sedative medications used, *%*	7.4	0
ApoE-ε4 allele carrier, *%*	24.9	2
English as native language, *%*	95.1	0
Smoking history,[3] *pack-years*	9.9 ± 16.7	16[2]
BMI, *kg/m²*	27.3 ± 4.2	0
Systolic blood pressure, *mm Hg*	137 ± 16.2	1[2]
Diastolic blood pressure, *mm Hg*	76.6 ± 9.7	0
Energy,[4] *MJ/d*	7.67 ± 2.35	3[2]
Alcohol,[4] *g/d*	13 ± 15	3[2]
Depression score[5]	8.1 ± 6.7	3[2]
Vigorous activity score[6]	7.9 ± 11.1	2[2]
Plasma glucose, *mmol/L*	5.65 ± 0.58	3[2]
Plasma α-carotene, *μg/mL*	0.09 ± 0.08	17[2]
Plasma β-carotene, *μg/mL*	0.32 ± 0.23	17[2]
Serum vitamin B-12, *nmol/L*	0.29 ± 0.18	8[2]
Serum folate, *nmol/L*	25.5 ± 9.33	7[2]
LDL cholesterol, *mmol/L*	3.16 ± 0.88	2[2]
Plasma homocysteine, *μmol/L*	10.6 ± 3.23	5[2]
HbA1c, *%*	5.78 ± 0.38	5[2]
Serum CRP, *mg/L*	3.79 ± 4.68	2[2]
Plasma MDA, *mmol/L*	0.20 ± 0.02	2[2]
Erythrocyte membrane FAs, *% of total FAs*		
DHA	4.99 ± 0.88	2[2]
EPA	0.88 ± 0.36	2[2]
DPA	2.30 ± 0.34	2[2]
Total n–3	8.18 ± 1.19	2[2]
Total fish intake,[7] *times/wk*	2.0 ± 1.4	0
Oily fish intake,[7] *times/wk*	0.8 ± 0.8	0
White fish intake,[7] *times/wk*	1.2 ± 1.0	0
Total fish intake,[7] *g/d*	40.2 ± 35.4	3
Historical fish intake,[8] *% eating fish ≥1 time/wk*		
Childhood	40.3	2
Early adulthood	67.0	5
Adulthood	71.0	5
Middle-age	79.0	0

[1] Values are means ± SDs or percentages. CCV, Cancer Council Victoria; CRP, C-reactive protein; DPA, docosapentaenoic acid; EPOCH, Older People Omega-3 and Cognitive Health; HbA1c, glycated hemoglobin; MDA, malondialdehyde; MMSE, Mini-mental State Examination.
[2] Missing data imputed.
[3] Pack-years = (number of cigarettes smoked/d × years of smoking)/20.
[4] Estimated from the CCV FFQ (29).
[5] From the Centre for Epidemiology Studies Depression Scale (31). Higher scores indicate more depressive symptoms with a maximum score of 60.
[6] From the Yale Physical Activity Survey (30). Unitless score; higher score is greater activity.
[7] Estimated from the PUFA FFQ (28), transformed from monthly intake.
[8] Based on a subset (n = 352) of total sample.

membrane proportions of DHA, EPA, and total n–3 PUFAs (r = 0.29–0.34; $P < 0.001$), but there was no association between erythrocyte membrane DPA and any of the fish consumption variables. Weekly white fish consumption exhibited lower correlations with DHA ($r = 0.18$; $P < 0.001$), EPA ($r = 0.13$; $P = 0.013$), and total n–3 PUFAs ($r = 0.16$; $P = 0.001$). Notably, the n–3 PUFA items assessing fish consumption frequency for tinned and fresh fish were moderately to strongly correlated with the 2 comparable items from the CCV FFQ ($r = 0.75$, $P < 0.001$ and $r = 0.45$, $P < 0.001$, respectively).

Current fish intake. Results from model 1, the initial adjusted model, for the full sample ($n = 390$) and subsample ($n = 352$) for the variables representing weekly consumption for oily, white, and total fish are presented in **Table 2**. The subsample represents those who provided historical fish intake information.

TABLE 2 Current fish consumption as a predictor of cognitive performance in EPOCH trial participants[1]

		Total fish intake		Oily fish		White fish	
Cognitive variable	Model	β[2]	*P*	β	*P*	β	*P*
Perceptual speed	Unadjusted	−0.05	0.31	−0.03	0.50	−0.04	0.46
	1[3]	−0.09	0.05	−0.07	0.17	−0.07	0.14
	1 (subsample)	−0.09	0.07	−0.07	0.20	−0.07	0.17
	3 (subsample)	−0.08	0.13	−0.04	0.42	−0.07	0.20
Psychomotor speed	Unadjusted	0.03	0.60	0.10	0.046	−0.04	0.38
	1	−0.03	0.58	0.07	0.17	−0.09	0.06
	1 (subsample)	−0.03	0.60	0.07	0.20	−0.09	0.08
	3 (subsample)	−0.05	0.31	0.05	0.31	−0.11	0.034
	Mediator entry[4]	—	—	—	—	−0.12	0.027
Inhibition[5]	Unadjusted	−0.12	0.016	−0.11	0.024	−0.07	0.19
	1	−0.11	0.028	−0.11	0.030	−0.05	0.33
	1 (subsample)	−0.10	0.05	−0.11	0.045	−0.04	0.34
	3 (subsample)	−0.19	0.037	−0.11	0.046	−0.05	0.35
	Mediator entry	−0.19	0.038	−0.11	0.049	—	—
Simple/choice reaction time	Unadjusted	−0.09	0.07	0.003	0.86	−0.12	0.015
	1	−0.13	0.008	−0.02	0.69	−0.15	0.002
	1 (subsample)	−0.13	0.013	−0.02	0.71	−0.15	0.003
	3 (subsample)	−0.20	0.027	−0.00	0.83	−0.15	0.005
	Mediator entry	−0.20	0.028	—	—	−0.15	0.004
Reasoning speed	Unadjusted	−0.07	0.16	−0.03	0.56	−0.07	0.17
	1	−0.10	0.045	−0.04	0.48	−0.10	0.043
	1 (subsample)	−0.10	0.06	−0.10	0.06	−0.04	0.49
	3 (subsample)	−0.10	0.07	−0.10	0.05	−0.02	0.66
Memory scanning speed	Unadjusted	−0.08	0.12	−0.07	0.12	−0.04	0.38
	1	−0.10	0.047	−0.07	0.13	−0.06	0.22
	1 (subsample)	−0.10	0.06	−0.07	0.16	−0.06	0.25
	3 (subsample)	−0.10	0.07	−0.07	0.22	−0.06	0.25
Working memory	Unadjusted	0.03	0.57	0.05	0.29	0.01	0.84
	1	−0.02	0.72	0.02	0.63	−0.03	0.52
	1 (subsample)	−0.02	0.74	0.02	0.65	−0.03	0.55
	3 (subsample)	−0.02	0.67	0.03	0.56	−0.04	0.43
Retrieval fluency	Unadjusted	0.06	0.21	0.08	0.13	0.04	0.48
	1	−0.01	0.86	0.02	0.60	−0.02	0.60
	1 (subsample)	−0.01	0.86	0.02	0.62	−0.02	0.62
	3 (subsample)	−0.01	0.81	0.03	0.48	−0.02	0.62
Short-term memory	Unadjusted	0.09	0.07	0.20	0.02	0.05	0.35
	1	0.02	0.66	0.07	0.15	−0.01	0.78
	1 (subsample)	0.02	0.68	0.07	0.17	−0.01	0.71
	3 (subsample)	0.01	0.87	0.06	0.23	−0.03	0.55
Reasoning	Unadjusted	0.00	0.83	0.04	0.43	−0.02	0.72
	1	−0.05	0.23	0.00	0.86	−0.07	0.10
	1 (subsample)	−0.05	0.27	0.00	0.82	−0.07	0.13
	3 (subsample)	−0.05	0.31	0.02	0.71	−0.08	0.13

[1] Current fish consumption assessed by the PUFA FFQ (28). Full EPOCH sample: n = 390; 388 with data for all variables. Subsample: n = 352; 339 with data for all variables. CCV, Cancer Council Victoria; EPOCH, Older People Omega-3 and Cognitive Health.

[2] β was the standardized regression coefficient; *P* was significant at <0.05 (2-tailed). *P* values were rounded to 2 decimal places or 3 when significant.

[3] Model 1 is adjusted for age, sex, years of education, and apoE-ε4 carrier status and tailored for each model, covariates that correlate with both the cognitive and fish variables at $P < 0.10$ from the potential covariates: smoking pack-years, English as native language, current income level, daily alcohol intake [g/d; CCV FFQ (29)], daily energy intake [MJ/d; CCV FFQ (29)], plasma α-carotene and β-carotene, serum vitamin B-12 and folate, vigorous activity level [Yale Physical Activity Survey (30)], and medication for hypertension, cholesterol, or cardiovascular conditions, as well as antidepressants, and/or sedatives. Model 3 is additionally adjusted for historical total fish consumption from all life periods.

[4] Mediating variables for individual models are presented in Supplemental Table 1.

[5] Reversed β sign for inhibition so that for all variables, a higher score equals better performance.

More frequent consumption of total fish (oily and white) was associated with slower cognitive speed for the constructs of inhibition, simple/choice reaction time, reasoning speed, and memory scanning. More frequent consumption of oily fish was significantly associated with worse inhibitory processes; similarly, consumption of white fish significantly and negatively predicted simple/choice reaction time. No sex or apoE-ε4 interactions were present in any models. Rerunning model 1 in the subsample with historical fish intake data for all cognitive variables essentially confirmed the previous findings with the

addition of the negative association between more frequent consumption of white fish and psychomotor speed. Controlling for historical total fish intake (25) in model 3 did not appreciably change the results. For the models with significant associations between the fish and cognitive variables, the potential mechanistic variables were entered into the models without the interaction variables [models 1 (entire and subsample) and 3]. All significant results remained.

Historical fish intake. Results from model 1 for the variables representing historical total fish consumption frequency are presented in **Table 3**.

Relations between historical total fish consumption frequency in childhood and cognitive function were significant for perceptual speed and simple/choice reaction time after controlling for all covariates in model 1 and remained significant in model 3. In contrast, no overall trend was apparent for the other life periods and only one association (psychomotor speed and total fish consumption frequency in middle-age) approached significance after adjusting for all covariates ($\beta = 0.12$; $P = 0.05$). No significant sex or apoE-ε4 interactions were present in any models.

Results from the alternative 3-category total historical fish consumption variable, with infrequent consumption as the reference group, were consistent with the binary variable, indicating consistent weak negative associations between greater fish consumption in childhood and the cognitive constructs.

n–3 FAs. The results from model 1, the fully adjusted model, for DHA, EPA, DPA, and total n–3 are presented in **Table 4**.

Analyses for DHA, DPA, and EPA found no associations with the cognitive constructs. However, an interaction between EPA and sex was obtained for both reasoning speed ($\beta = -0.18$; $P = 0.005$) and perceptual speed ($\beta = -0.15$; $P = 0.023$) (model 2). (Females were coded as 1 and males as 0.) As shown in **Figure 1**, as percentage of EPA increased, speed scores increased for males

TABLE 3 Historical total fish consumption as a predictor of cognitive performance in the reduced sample of EPOCH trial participants[1,2]

		Total fish intake childhood		Total fish intake early-adulthood		Total fish intake adulthood		Total fish intake middle-age	
Cognitive variable	Model	β[3]	P	β	P	β	P	β	P
Perceptual speed	Unadjusted	−0.06	0.28	0.03	0.53	0.05	0.37	−0.01	0.91
	1[4]	−0.11	0.027	−0.01	0.90	0.02	0.64	−0.03	0.59
	3	−0.11	0.037	0.00	0.96	0.07	0.24	−0.02	0.76
Psychomotor speed	Unadjusted	0.01	0.83	0.06	0.23	0.07	0.18	0.12	0.023
	1	−0.03	0.56	0.02	0.62	0.05	0.31	0.10	0.044
	3	−0.05	0.37	0.00	1.00	0.01	0.77	0.12	0.05
Inhibition[5]	Unadjusted	−0.08	0.12	0.02	0.73	0.04	0.50	0.04	0.41
	1	−0.10	0.08	0.01	0.90	0.04	0.44	0.04	0.48
	3	−0.09	0.10	0.00	0.93	0.05	0.44	0.07	0.23
Simple/choice reaction time	Unadjusted	−0.10	0.07	0.05	0.38	0.03	0.62	0.00	0.96
	1	−0.12	0.019	0.01	0.82	0.00	0.92	−0.02	0.72
	3	−0.12	0.029	0.04	0.52	0.03	0.60	0.01	0.85
Reasoning speed	Unadjusted	−0.10	0.05	0.01	0.79	0.06	0.26	0.03	0.61
	1	−0.10	0.09	−0.00	0.96	0.03	0.51	0.01	0.82
	3	−0.10	0.11	−0.01	0.91	0.06	0.33	0.02	0.70
Memory scanning speed	Unadjusted	−0.09	0.08	0.01	0.82	0.02	0.73	0.03	0.54
	1	−0.10	0.06	0.01	0.89	0.00	0.92	0.02	0.68
	3	−0.10	0.08	0.00	0.95	0.02	0.78	0.06	0.34
Working memory	Unadjusted	0.00	0.93	0.07	0.23	0.10	0.06	0.05	0.32
	1	−0.05	0.31	0.01	0.76	0.06	0.22	0.02	0.65
	3	−0.06	0.24	−0.01	0.89	0.08	0.16	0.00	0.96
Retrieval fluency	Unadjusted	0.01	0.82	0.03	0.59	0.02	0.69	0.05	0.34
	1	−0.08	0.10	−0.02	0.58	−0.01	0.82	0.02	0.66
	3	−0.08	0.12	−0.02	0.67	0.00	0.99	0.04	0.48
Short-term memory	Unadjusted	0.06	0.22	0.07	0.20	0.09	0.11	0.10	0.06
	1	−0.02	0.71	0.01	0.82	0.05	0.35	0.07	0.15
	3	−0.03	0.51	−0.02	0.70	0.04	0.50	0.05	0.33
Reasoning	Unadjusted	−0.02	0.71	0.03	0.58	0.04	0.50	0.04	0.46
	1	−0.09	0.08	−0.02	0.69	0.00	0.96	0.01	0.86
	3	−0.08	0.11	−0.02	0.77	0.02	0.73	0.03	0.58

[1] Historical fish intake assessed by the LDQ (24); total fish intake is coded as 0 (rarely/never and 2–3 times/mo) or 1 (once a week or more). CCV, Cancer Council Victoria; EPOCH, Older People Omega-3 and Cognitive Health.
[2] Reduced sample: $n = 352$; 339 with data for all variables.
[3] β was the standardized regression coefficient; P was significant at <0.05 (2-tailed). P values were rounded to 2 decimal places or 3 when significant.
[4] Model 1 is adjusted for age, sex, years of education, and apoE-ε4 carrier status and tailored for each model, covariates that correlate with both the cognitive and fish variables at $P < 0.10$ from the potential covariates: smoking pack-years, English as native language, current income level, early-life income level (for the childhood and early-adulthood models), daily alcohol intake [g/d; CCV FFQ (29)], daily energy intake [MJ/d; CCV FFQ (29)], vigorous activity level [Yale Physical Activity Survey (30) and the Lifetime Diet Questionnaire (24)], and medication for hypertension, cholesterol, or cardiovascular conditions, as well as antidepressants, and/or sedatives. Model 3 is additionally adjusted for historical total fish consumption in all other life periods and for contemporary intake.
[5] Reversed β sign for inhibition so that for all variables, a higher score equals better performance.

TABLE 4 Erythrocyte membrane DHA, EPA, DPA, and total n–3 PUFA percentages as a predictor of cognitive performance in EPOCH trial participants[1]

		DHA		EPA		DPA		Total n–3	
Cognitive variable	Model	β[2]	P	β	P	β	P	β	P
Perceptual speed	Unadjusted	−0.07	0.17	0.00	0.96	−0.03	0.53	−0.06	0.24
	1[3]	−0.08	0.10	−0.04	0.43	−0.02	0.67	−0.07	0.14
	3	−0.06	0.28	−0.01	0.77	−0.02	0.62	−0.05	0.36
Psychomotor Speed	Unadjusted	0.05	0.30	0.07	0.14	0.00	0.96	0.06	0.23
	1	0.04	0.39	0.04	0.39	0.01	0.79	0.05	0.26
	3	0.06	0.27	0.05	0.29	0.01	0.80	0.07	0.17
Inhibition[4]	Unadjusted	0.00	0.94	0.04	0.47	−0.06	0.25	−0.01	0.76
	1	0.003	0.95	0.05	0.30	−0.02	0.73	0.02	0.73
	3	−0.04	0.41	0.09	0.08	−0.02	0.64	0.06	0.24
Simple/choice reaction time	Unadjusted	−0.07	0.19	0.04	0.46	−0.03	0.59	−0.05	0.32
	1	−0.05	0.27	0.01	0.84	−0.04	0.44	−0.04	0.36
	3	−0.01	0.82	0.05	0.32	−0.04	0.40	−0.00	0.92
Reasoning speed	Unadjusted	−0.06	0.26	0.20	0.99	0.00	0.96	−0.05	0.36
	1	−0.06	0.22	−0.20	0.70	−0.05	0.33	−0.07	0.07
	3	−0.03	0.55	0.05	0.92	−0.05	0.30	−0.04	0.43
Memory scanning speed	Unadjusted	−0.10	0.04	0.00	0.91	−0.03	0.57	−0.08	0.10
	1	−0.09	0.08	0.00	0.92	−0.03	0.47	−0.07	0.16
	3	−0.06	0.26	0.02	0.64	−0.04	0.43	−0.04	0.41
Working memory	Unadjusted	−0.01	0.90	0.06	0.22	0.00	0.96	0.01	0.86
	1	−0.04	0.42	0.00	0.98	−0.02	0.59	−0.04	0.45
	3	−0.03	0.54	0.01	0.83	−0.02	0.58	−0.03	0.57
Retrieval fluency	Unadjusted	0.01	0.87	0.09	0.07	−0.00	0.91	0.02	0.76
	1	−0.07	0.18	0.01	0.78	0.00	0.99	−0.05	0.32
	3	−0.07	0.12	0.01	0.75	0.00	1.00	−0.05	0.32
Short-term memory	Unadjusted	0.04	0.44	0.12	0.017	−0.20	0.75	0.05	0.28
	1	−0.01	0.89	0.06	0.31	−0.01	0.80	0.01	0.87
	3	−0.01	0.77	0.05	0.36	−0.01	0.81	0.00	0.98
Reasoning	Unadjusted	−0.04	0.48	0.06	0.27	0.01	0.91	−0.01	0.80
	1	−0.06	0.17	0.00	0.99	−0.02	0.67	−0.05	0.25
	3	−0.05	0.29	0.01	0.77	−0.02	0.65	−0.04	0.40

[1] Full EPOCH sample: n = 390; 388 with data for all variables. CCV, Cancer Council Victoria; DPA, docosapentaenoic acid; EPOCH, Older People Omega-3 and Cognitive Health.
[2] β was the standardized regression coefficient; P was significant at <0.05 (2-tailed); P values were rounded to 2 decimal places or 3 when significant.
[3] Model 1 is adjusted for age, sex, years of education, and apoE-ε4 carrier status and tailored for each model, covariates that correlate with both the cognitive and FA variables at $P < 0.10$ from the potential covariates: smoking pack-years, English as native language, current income level, daily alcohol intake [g/d; CCV FFQ (29)], daily energy intake [MJ/d; CCV FFQ (29)], plasma α-carotene and β-carotene, serum vitamin B-12 and folate, vigorous activity level (30), and medication for hypertension, cholesterol, or cardiovascular conditions, as well as antidepressants, and/or sedatives. Model 3 is additionally adjusted for current fish intake.
[4] Reversed β sign for inhibition, so that for all variables, a higher score equals better performance.

but decreased for females. Trends for males were not significant (perceptual speed: $\beta = 0.06$, $P = 0.37$; reasoning speed: $\beta = 0.10$, $P = 0.12$) but were for females ($\beta = -0.16$, $P = 0.028$ and $\beta = -0.17$, $P = 0.021$, respectively). These results remained unchanged after controlling for potential mechanistic variables.

To examine any potential impact of fish intake as a confounding factor on these results, current total weekly fish intake was entered into model 2 as a covariate. Male trends remained similar and trends for females, although still negative, were no longer significant (perceptual speed: $\beta = -0.14$, $P = 0.06$ and reasoning speed: $\beta = -0.14$, $P = 0.05$). The interaction effects for sex and EPA remained significant and of a similar magnitude.

Discussion

The findings of this study do not support the hypothesis that higher erythrocyte membrane n–3 PUFA proportions or fish intake are associated with better cognitive performance. Instead, they suggest a weak negative association between historical and current fish consumption and cognitive speed. The absence of significant relations between blood concentrations of n–3 PUFAs and cognitive performance across a number of domains, after controlling for fish intake, is consistent with the cross-sectional analyses reported by the Folic Acid and Carotid Intima-media Thickness trial (9).

Higher concentrations of EPA were associated with poorer perceptual and reasoning speed in females, but not in males, before controlling for current fish intake, a relation not previously reported. Although Whalley et al. (16) reported evidence for a sex-based differential effect of total n–3 FAs on cognition over time, the nature of this difference was not elaborated. In a recent trial, van de Rest et al. (56) showed a benefit of fish oil supplementation only in males, which is not inconsistent with the current findings. Some evidence exists for a sex-based differential response in lipid profiles to fish oil supplementation, including a greater TG-lowering action of high-dose (but not low-

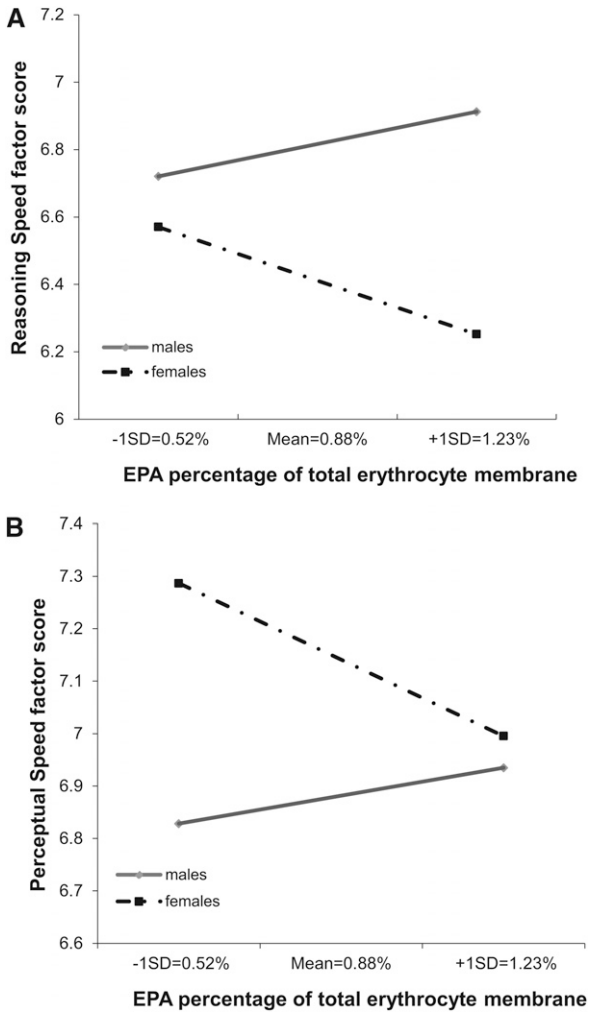

FIGURE 1 Significant interactions between sex and erythrocyte membrane EPA predicted reasoning speed and perceptual speed in EPOCH trial participants. Regression models were adjusted for age, sex, years of education, apoE-ε4 carrier status, and covariates that correlated with both the cognitive and FA variable at $P < 0.10$. For reasoning speed (*A*), the additional variables were income level and daily alcohol intake [g/d; CCV FFQ (29)]. For perceptual speed (*B*), the additional variables were income level, English as native language, and plasma homocysteine. Higher factor scores indicated faster/better performance, $P < 0.05$. CCV, Cancer Council Victoria; EPOCH, Older People Omega-3 and Cognitive Health.

dose), long-chain n–3 PUFAs in males (57). It is plausible that physiologically different actions of long-chain n–3 PUFAs in the sexes lead to differential associations with cognition.

The aforementioned negative association of EPA seen in females, and the worse cognitive performance in those with greater fish consumption, is consistent with 2 other studies (11,13). The comprehensive approach to assessing cognitive function in the current study has not been used in other relevant studies. The use of factor scores derived from Confirmatory Factor Analysis means our cognitive scores reflect only construct-related variance (not error variance or variance specific to a particular task), leading to greater power with which to detect an effect, even over composite or factor-based scores (58). Supporting this notion, when using cognitive domain scores comprising more than one task, a trend toward worse performance on a language/memory component score was found with increasing n–3 PUFA and fish intake in elderly men (13) and was not observed when using the individual task scores as dependent variables. Another finding of a negative relation was observed in prospective analyses of older adults aged 65 y and older in which higher plasma EPA was found in those who developed cognitive impairment compared with unimpaired controls, and higher proportions of DHA and total n–3 were found in the dementia cases compared with the controls (11).

The significant negative associations with fish intake herein were all with cognitive speed constructs. Although other studies have included a composite general cognitive speed measure and not found this negative relation (13,15), cognitive speed is also multifaceted (59–62) and associations may not be captured by a general speed measure.

Potentially, the negative trends reported here between fish consumption and erythrocyte membrane EPA concentrations (in females) may be due to neurotoxic contaminants in fish, such as methylmercury (38,63). Most negative associations between historical fish consumption and cognition were for childhood, a time when the developing nervous system is sensitive to the neurotoxic effects of even low concentrations of mercury (63). Greater contemporary fish intake was also related to worse cognitive performance and the aging brain may be more sensitive to the effects of methylmercury (64). Potentially, any beneficial effects of the long-chain n–3 FAs may be masked by detrimental effects of contaminants, such as mercury, in fish (65). However, although controlling for current fish consumption attenuated the negative relation between EPA and cognitive speed seen for females, no significant positive relations between n–3 FAs and the cognitive constructs emerged.

A limitation of the current study is that we did not measure methylmercury. However, studies have confirmed a relation between blood mercury concentrations in humans and fish intake (66), n–3 PUFA intake (38), and plasma n–3 concentrations (65), so further investigation of the possibility that the negative trends observed here between cognitive performance and fish consumption were due to neurotoxicity seems warranted. Our sample size was not large but compared favorably with studies that found positive relations. The mean frequency of fish consumption in the group we studied was twice per week and therefore comparable with a group in which a positive effect of fish intake on normal, older-age cognition was found (15). In addition, the n–3 concentrations in the current sample were either similar or lower than those in other studies that found positive effects (8,16); the lack of effect was thus not due to our sample having very high proportions of n–3 FAs. However, our sample consisted of predominantly Caucasian, relatively high-functioning, community-dwelling older adults, so our results are not necessarily generalizable to other populations.

In conclusion, we have found no evidence of a beneficial effect of increased long-chain n–3 FA concentrations or fish intake on baseline cognitive performance in cognitively normal older adults from the EPOCH trial. Instead, the results suggest a small negative effect of fish intake in childhood and older age on older-age cognitive function, which warrants further investigation. This is a novel finding, so replication is necessary in other normally functioning populations. When assessing associations between current fish intake and cognitive functioning, gathering more detailed information regarding the type of fish consumed could inform the investigation of any potential negative effects of fish. Also, longitudinal associations between past fish intake and cognition should be examined to assess the impact, if any, that past consumption has upon the trajectory of cognitive change in normally functioning older people.

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